CHAPTER 4

Joseph S. Wall Charles W. Blessin

Composition of Sorghum Plant and Grain¹

INTRODUCTION

As cultivation of sorghum became widespread, different varieties were selected for specialized uses. Sweet sorghums were bred for the sugar contained in their stems and for succulence for use as forages. Among varieties, the grain may differ in amount, color, size, and chemical composition. During plant development, changes in composition occur that are important in selecting the times of cropping for forage or other uses. When compared to other grain or forage crops, sorghums contain distinctive components that offer advantages or may require special consideration during utilization. Therefore an evaluation of a range of compositions related to varieties and hybrids, conditions of cultivation, and time of cropping is essential to select materials for optimum specific uses as food, feed, or fiber. This chapter will discuss some factors which influence the composition of plants and grains of various types of sorghums and will describe some of the characteristics of their constituent carbohydrates, proteins, lipids, pigments, minerals, enzymes, and other substances.

PROXIMATE ANALYSIS

Proximate analysis of plant materials consists in determining the major classes of chemical components, which include moisture, crude protein, crude fat, fiber, ash, and nitrogen-free extract. Protein in many feed materials, including sorghum, is approximated by multiplying the Kjeldahl nitrogen analysis by the factor 6.25. Crude fat is measured as diethyl ether or petroleum ether extractable material. Crude fiber refers to combustible organic matter not solubilized by either hot dilute sulfuric acid or dilute sodium hydroxide solutions. Ash is determined by igniting samples until free of carbon. Nitrogen-free extract is the difference between the sum of these constituents and the original dry sample weight. Rapid, reproducible, uniform methods are published by the Association of Official Agricultural Chemists (1965) and by the American Association of Cereal Chemists (1962). Proximate analysis

¹ Contribution from the Northern Regional Research Laboratory, Peoria, Illinois. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Dept. of Agr.

provides a good initial impression of the relative nutritive value and utility of an agricultural commodity, and allows a basis of comparison between different species, plant parts, and cultivation conditions. However, based on his studies, Van Soest (1967) cautions against using proximate analysis data as the only criteria for feed value.

Proximate Analysis of Forage

Variations Due to Varieties and Hybrids.—The composition of hybrid forage sorghums favors their use as fodder, silage, and other feed components for ruminants in many areas where they provide better yields than corn. Corn and sorghum forage compositions are fairly similar, although greater grain content in the corn forage accounts for its slightly higher levels of crude protein and lower contents of crude fiber than sorghum forages (Table 4.1).

Forage	Dry Matter, %	Crude Protein, %	Crude Fat, %	Crude Fiber,	$_{\%}^{\mathrm{Ash,}}$	Nitrogen- free Extract,	Reference
Corn	36.1	8.34	3.52	26.4	5.61	56.1	1
Sorghum Atlas	24.25	5.4	4.4	27.3	8.07	54.8	1
FS-1A	24.6	7.3	$\hat{2}.\hat{9}$	23.6	9.72	56.4	1
FS-22 Brawley	24.3	6.7	4.8	32.8	12.8	0012	1
(sweet forage)	27.4	6.2	2.5	20.6	7.6		2

Table 4.1
CHEMICAL ANALYSIS OF FORAGE SORGHUMS AND CORN

In general, the more grain that varieties of sorghum will yield, the more starch, lipid, and protein the entire plant will contain along with less fiber. In Table 4.1 are compared analyses of plants from DeKalb FS-1A, a high-grain-yielding forage hybrid; FS-22, a low grain producer; and Atlas, an intermediate grain producer. Nordquist and Rumery (1967) found that FS-1A contains significantly less fiber than FS-22, whereas Atlas is intermediate in fiber content. Ramsey $et\ al.\ (1961)$ noted that forage of RS 610, a short combine type of hybrid grain sorghum, has only slightly less fiber (28%) as compared to that of Tracy, a sweet sorghum (30%), but significantly more protein (11 versus 5%).

Another factor that greatly influences the composition of sorghum forage is the relative amount of leaf and stalk in the plant since leaves

¹ Nordquist and Rumery (1967).

² Garret and Worker (1965).

are higher in crude protein and fat than stalk. Table 4.2 compares composition of plant parts of Atlas forage (Stallcup *et al.* 1964). Thurman *et al.* (1964) showed that the better leafage in new sorghum hybrids improves their protein content.

The ratio of leafage to stalk also influences the level of nutrients in sudangrass. Gangstad (1964) found in a series of sudangrass varieties positive correlation between leafiness and protein and fat content, and an inverse relationship between fiber and leaf content.

Effect of Development and Agronomic Factors.—The composition of forage sorghums has been determined at various stages of plant development to establish systems of crop management that give optimum yields of nutrients (Webster and Davies 1956; Eilrich et al. 1964; Webster 1963; Bettini and Proto 1960). The greatest yield of total forage

			Dry 1	Iatter		
Forage Material	Flant,	Protein,	Ether Extract,	Crude Fiber,	Ash,	Nitrogen- free Extract,
Atlas			,			
Whole plant	100.0	5.7	1.9	23.6	4.7	64.1
Leaves and sheath	21.2	7.7	3.1	29.9	8.2	51.1
Stalks	55.1	3.0	0.8	26.1	3.9	66.2
Head	23.7	10.0	3.5	12.2	3.4	70.9

Source: Stalleup et al. (1964).

from Atlas sorghum comes during the hard dough stage of grain development. Ash ranges from 8 to 11% in young plants (30 in.), but after heading decreases to 4 to 5%. Ether extract will vary between 1 and 2%. In early stages of growth, protein accounts for 12 to 18% of dry weight, but drops to 5 to 8% as the plant reaches maturity. The decline in protein is most marked in the leaves and stem. Protein content of heads initially increases rapidly but levels off (Eilrich et al. 1964). Maximum protein yield does not coincide with maximum forage yield, but occurs earlier at the soft dough stage of grain. Fiber content decreases from a level of around 35% in the young plant to a minimum, 24%, during heading, and then rises slightly as maturity is reached. The nitrogen-free extract rises from a low value of 45% to about 60% when the grain is formed.

Degree of plant maturity also affects the quality and composition of sudangrass and sorghum-sudangrass hybrids for use in grazing or

hay. Stallcup *et al.* (1964) determined proximate analysis of Piper Sudan grass forage cut at different stages of growth. There is a steady decline in protein content, 17% at 18- to 24-in. height, as compared to 10% at early head. Ether extract declines from 3.8 to 2.9%, and ash drops from 11.1 to 6.3% at the same stages of harvest. Crude fiber increases from 24 to 35%.

After extended weathering in the field after frost, a significant loss in dry matter in forage sorghum occurs, primarily in nitrogen-free extract (Webster and Davies 1956). Little change in percentage of protein was observed, but a significant increase in relative amount of fiber occurred. Burns and Wedin (1964) state that during after-frost sampling of sudangrass, there was little loss in dry matter.

The composition of the sorghum plant depends on the conditions of cultivation, including such factors as soil, fertilizer, climate, and plant population. Eilrich et al. (1964) found that when area per plant is decreased, the dry weight per plant declines but total dry matter per acre increases. Protein and nitrogen-free extract diminish and fiber increases in crowded plant plots. Quality, especially protein content, and yield can be improved by increased fertilizer application provided that moisture and other factors are adequate (Burleson et al. 1959).

Proximate Analysis of Grain

Variations in Varieties and Hybrids.—The composition of sorghum grain is comparable to that of other grains grown extensively for food and feed. Like corn and wheat, sorghum grain is low in fiber and ash, because the glumes are readily removed from most varieties. The protein level of sorghum grain is slightly higher than corn or rice. The oil content of sorghum is lower than in corn or oats, but is higher than in rice, wheat, or barley. The ash content of sorghum is lower than in cereals that contain attached glumes. Sorghum grain ranks next to corn in amount of total available energy among common cereal grains.

The composition of sorghum grains from grain, syrup, and forage varieties was extensively investigated in several laboratories (Heller and Sieglinger 1944; Barham et al. 1946; Edwards and Curtis 1943). Ash ranged from 1.6 to 2.2%; oil varied from 3.1 to 4.9%; protein values, from 11 to 15%. In general, grain sorghum kernels had higher starch and lower fat contents than those of the smaller seeds of forage or syrup varieties. In a large number of waxy and normal grain sorghum selections analyzed by Horan and Heider (1946) no major differences were found in general composition.

With the development of improved grain sorghum varieties and

hybrids, irrigation, and fertilization, the grain has become larger, better filled with starch, and has a reduced protein content. In Table 4.3 are tabulated the analyses of some hybrids and varieties grown in Guatemala (Bressani and Rios 1962). These data are expressed on a 14% moisture basis. Ether extract presented no large differences among hybrids or varieties, ranging from 3.1 to 4.5%. Crude fiber was rather consistent. Ash ranged from 1.4 to 3.9%; protein varied considerably, from 7.6 to 12.5%.

Sample	Protein,²	Ether Extract, %	Crude Fiber,	$rac{ ext{Ash}}{\%}$	Calcium, Mg %	Phosphorus, Mg %
Hybrids						
RS 620	8.5	4.4	2.7	3.42	16.45	595
RS~610	7.6	3.7	2.6	2.97	19.23	542
H11X71	10.4	3.♀	2.0	1.39	22.31	238
Varieties						
Combine						
kafir-60	8.1	3.5	2.9	2.77	13.48	536
Caprock	10.0	3 . 4	3.0	3.07	21.19	512
Westland	9.0	3.3	2.6	3.73	45.53	1,097
Norghum	9.0	3.9	2.8	2.43	17.18	479
Martin	8.5	3.1	2.1	2.09	14.59	373
Hegari	8.2	3.5	2.5	2.33	17.12	416

 $^{^1}$ All values expressed on 14% moisture basis after Bressani and Rios (1962).

² Protein = % N \times 6.25.

The variation in grain composition may result from differences in composition of the different parts of the grain; bran, germ, or horny and floury endosperm. Bidwell et al. (1922) hand separated the parts of three sorghum grains-kafir, Dwarf milo, and feterita-and determined their amounts and compositions. Among varieties the germ varied from 7 to 11% of the kernel, and bran from 6 to 7%. Considerable differences occurred in levels of horny endosperm which ranged from 49 to 61%. As shown in Table 4.4, Dwarf milo grain germ had a high content of oil, protein, and ash; 70% of the oil, 15% of the protein, and 20% of the ash of this grain were in the germ. Next to germ, the horny endosperm had the highest content of protein. Endosperm contained less ether extract and ash than other fractions. Starchy endosperm was richer in ether extract than horny endosperm. The pericarp or bran fraction accounted for most of the fiber in the grain and contained some starch. Similar results were reported by Hubbard et al. (1950) on analyses conducted on grain parts from varieties of grain sorghum current at that time.

Component Part of Kernel	Proportion of Kernel,	Ash,	Ether Extract,	Protein, %	Crude Fiber,	Nitrogen-free Extract, %	Starch,
Whole grain	100.0	1.89	3,47	13.99	1.93	78.79	68,52
Bran	5.5	3.07	4.33	7.08	15.36	70.16	1.60
Horny endosperm	54.7	0.56	0.15	15.11	0.69	83.49	72.24
Starchy endosperm	28.7	0.71	0.28	8.91	0.81	89.29	82.5
Germ	11.1	9.46	19.99	20.84	9.11	40.67	1.53

Source: Bidwell et al. (1922).

Kersting et al. (1961) followed chemical changes in developing sorghum grain. The nitrogen content decreases slightly during the first 10 days after pollination and then remains constant at about 2 to 3%. During germination, sorghum grain changes in composition. Aucamp et al. (1961) found that in the sprouted grain used for malt there is a decrease in fat and carbohydrate; protein remains the same or increases slightly.

Agronomic Factors.—Soils and weather influence the yield and chemical composition of grain sorghums. Heller and Sieglinger (1944) noted in years of drought and high temperature that the yields of sorghum decreased and that the protein level increased at the expense of starch and fat. Location greatly influenced variation in composition. Miller et al. (1964) conducted tests on different hybrids at eight Kansas locations. For single hybrids, such as RS 610, the protein content ranged from 7 to 10% at the test locations.

Crop management also is important in determining grain composition. As shown by Miller et al. (1964) grain that was grown on nonirrigated land, continuously cropped, had an average yield of 2.0 tons per acre and 9.5% protein. In contrast, irrigated land produced 3.3 tons of grain per acre at a protein content of 8.3%. Miller et al. (1964) and Burleson et al. (1956) demonstrated that protein content and yield of grain can be increased by nitrogen fertilization.

CARBOHYDRATES OF THE SORGHUM PLANT

Sugars and starches are the main storage forms of energy in the sorghum plant. Cellulose and hemicellulose contribute to structural components in the plant.

Sugars in Stem and Leaf

Glucose and fructose are the predominant monosaccharides and reducing sugars in stem and leaf. Sucrose, a nonreducing sugar, is the major disaccharide. Sugars are generally determined directly or on hydrolyzates of alcoholic extracts of plant materials by their reducing action on copper or ferricyanide solutions. Smith (1962) used paper chromatography to separate and determine sucrose, glucose, fructose, and maltose in extracts of sorghum leaves and stems.

Varietal Variations.—Sugars in the stem are important commercially as a source of syrup and contribute to forage quality. The quantity and composition of juices and sugars in mature stem vary with the

variety or the hybrid. Sweet sorghum fodders may contain 21% total sugars on a dry basis, whereas grain sorghum fodders have only 5 to 6%.

Extensive studies of the composition of sorghum juices from stems of varieties produced for syrup or sugar have provided detailed information on the sugar content of these plants (Webster *et al.* 1954; Ventre *et al.* 1948). Sucrose, the major sugar in the stalk juice of the ripe plant, ranges from 6 to 15% with most varieties at 13%. Glucose ranges from 0.5 to 5% and fructose from 0 to 1.5%. According to Eilrich *et al.* (1964) in mature plants of Atlas sorghum, a forage variety, the level of reducing sugars exceeds sucrose.

Sugar contents of sudangrass varieties were correlated with grazing preference. For the 10 varieties and hybrids tested by Gangstad (1964), total sugar content varied from 8 to 15%; reducing sugars ranged from 2 to 4%.

Variations in Sugar During Development.—The content of the different sugars varies as the plant develops. The increase in total sugars in the plant between the dough to ripe stages is nearly double that between the milky and dough stages. In the very young plant (40-45 days) reducing sugars are highest in concentration (Webster et al. 1948). Ventre et al. (1948) compared sugar contents of stalk juice at three stages of growth of the plant. In the early stages the fructose concentrations were high and exceeded that of glucose in some varieties. In most sweet sorghums the sucrose level increases in the stem until maturity (Webster 1963). In contrast, in Atlas the sugar content of the stems may decline slightly during seed formation (Eilrich et al. 1964). Grain sorghums in early growth may have higher levels of sugars in stems and leaves than sweet sorghums (Webster et al. 1948). However as the grain sorghums set heads, the sugar content of the stem declines precipitously. Sterile varieties in which the setting of seed is impaired have elevated sugar contents in forage parts (Webster 1963).

Regions of the stalk differ in sugar content; the center portion is the richest in sugars. Ventre *et al.* (1948) noted that lower portions of the stem contain more glucose than sucrose. Sugar content and succulence of stem are reduced by crowding of plants (Eilrich *et al.* 1964).

Sugars increase greatly, from 3 to 9%, in the leaves during the period 2 to 3 weeks after bloom of Atlas sorghum. A slight decrease in leaf sugar occurs as starch is deposited in the grain (Eilrich *et al.* 1964). Also, sugar content in leaves of sorgos and milos is subject to diurnal variation reaching a maximum in the afternoon and gradually decreasing until daylight of the next morning (Miller 1924).

Starch in Stem and Leaves

Starch is present in the leaves and stems of all sorghum varieties. In Atlas sorghum leaves, acid-hydrolyzable carbohydrates rise to 25% of dry weight shortly after bloom (Eilrich et al. 1964). They diminish considerably as the grain develops. In stems, starch rises to about 16% but diminishes also during grain formation. In Atlas sorghum, starch is deposited in the culm after the grain is mature. Small amounts of starch are extracted along with stalk juices from sorgo varieties (Sherwood 1923). Sudangrass and sudangrass-sorghum hybrids contain starch in leaves and stems at all phases of growth (Gangstad 1964).

Hemicellulose in Stem and Leaf

Hemicelluloses are major components of plant cell walls and of fibrous and parenchymal tissues. The hemicelluloses may consist of pentoses, such as xylose and arabinose; sugar acids, such as glucuronic and galacturonic; and hexose sugars, such as glucose and galactose; all linked by β -1,4-bonds. Hemicelluloses are extracted by strong alkali from tissues freed of sugars and starches. Hemicelluloses are chemically different from the α - or regular cellulose which is insoluble in strong alkali. Pentosans are hemicelluloses composed primarily of pentoses. They are determined by boiling tissues in concentrated hydrochloric acid and measuring the furfural evolved.

Pentosan contents of stems of several varieties of sorghums are summarized in Table 4.5. Lengyel and Annus (1960) found that sweet sorghums have the lowest content of pentosans (20%), whereas in grain sorghums, broomcorn, and sudangrass, pentosans are higher. Gangstad (1964) found that pentosan content in several sudangrasses ranges from 20 to 23%.

The heads of grain sorghums are especially rich in pentosans. Pento-

Type of Sorghum	Cellulose, %	Pentosan, %	Lignin, %	$\begin{array}{c} \text{Alcohol-Benzene} \\ \text{Extract,} \\ \% \end{array}$
Grain sorghums	29	24	16	7
Broomcorns	39	24	15	8
Sweet sorghums	26	17	10	25
Sudangrass	43	45	16	4
Johnsongrass	42	27	17	5

Source: Lengyel and Annus (1960).

sans constitute 32.8% of glumes or hulls removed from Leoti sorghum (Edwards and Curtis 1943).

In the course of extracting sugars from sweet sorghum, a methanolinsoluble material was recovered which inhibited crystallization of sugars (Harris *et al.* 1952). Hydrolyzates of this hemicellulose gum yielded four sugars: glucose, galactose, arabinose, and xylose.

Cellulose in Plant

Cellulose is the major component of cell walls and is responsible for most of the strength of fibrous tissues. Cellulose is a polymer of glucose, but unlike starch which has α -1,4 and α -1,6 bonds, the glucose molecules are linked exclusively by β -1,4 bonds. Crude cellulose is generally determined as the residue of chlorinated pulps insoluble in sodium sulfite. Crude cellulose may contain some pentosan and its analysis closely parallels the crude fiber analysis of sorghum forage hybrids (Stallcup *et al.* 1964; Proto 1962).

Variations in Cellulose Contents.—The crude cellulose analysis of different types of sorghums is given in Table 4.5 (Lengyel and Annus 1960). Sweet sorghum varieties contain less cellulose than grain sorghums or broomcorn varieties. Also, mature sudangrass and johnsongrass exceed sweet sorghums in cellulose content. Proto (1962) reports that 4 forage hybrids (Camelsorgo, Beefbuilder, Siloking, and S_{11}) contain about 29% crude cellulose. Leafy forage hybrids range from 24 to 27% cellulose (Stallcup *et al.* 1964).

The content of crude cellulose does not vary greatly during the development of the plant (Bettini and Proto 1960). The content of cellulose is higher in the stem than the leaf. The stalk rind is higher in cellulose (34%) than the pith (19%) (Stallcup *et al.* 1964).

Sorghum Cellulose for Paper.—The amount and quality of cellulose in sorghum stalks have encouraged their investigation as possible pulp sources for the paper industry. The α -cellulose in sweet sorghum stem residues from syrup manufacture accounts for 35% of the dry weight; pentosans, 27%; and lignin, 20% (Sorgato 1949). A good yield of crude cellulose suitable for pulp is obtained upon treatment of the ground-pressed stalks with 2 to 5% NaOH at 120°C for 3 hr. Seventy-five percent of the lignin is removed in such processing.

Escourrou (1959) concluded that the bagasse from sugar sorghums was not the most productive sorghum source of cellulose because of lower yield and transportation, storage, and processing problems. He investigated the yields of fiber obtained directly from numerous sorghums. Several varieties of broomcorn sorghum, principally of the

Evergreen type, yielded excellent pulps in good quantity. Johnsongrass, while yielding lesser quantities of cellulose was desirable because of its perennial regrowth. The broomcorn was planted at a high density population and produced 7 to 11 tons of crude cellulose per acre.

Fibers of sorghum pulps are shorter in length $(2,000~\mu)$ than those of coniferous and hard woods $(3,000~\mu)$, but are longer than those of other leafy species $(1,000~\mu)$ (Escourrou 1959). They have smaller diameters, $16~\mu$, as compared to $40~\mu$ for pine fibers. The high length-to-diameter ratio permits sorghum fibers to serve as a reinforcement agent for other fibers in paper as they fill interstices well. X-ray examination establishes that even after sulfite processing, the cellulose of sorghum fiber retains a high degree of molecular orientation. Consumption of soda and chlorine bleaches is low for preparing sorghum pulps. Burst resistance, folding strength, and other characteristics of papers fabricated from pulps of sorghums of various types are satisfactory (Lengyel and Annus 1960; Escourrou 1959).

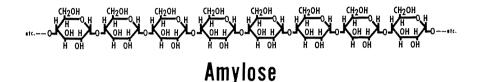
Starch in Sorghum Grain

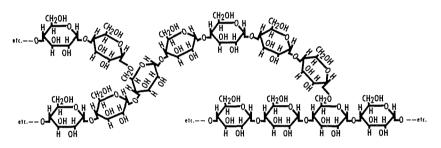
Starch Analyses.—Cereal grains including sorghum are valued for their high content of energy in the form of starch. Starch content in defatted sorghum grain meals may be determined as reducing sugar after enzyme and acid hydrolysis. Also, the starch may be solubilized and determined by its rotation of polarized light. The specific rotation, $[\alpha]_D$, of sorghum starch is 203.1 to 203.5, whereas that for wheat starch is 202.7 to 203.2 (Patel *et al.* 1960). Using these procedures, Edwards and Curtis (1943) found that starch constituted 68 to 73% of the grain from 20 varieties of grain and syrup sorghums. The starch content was highest in milos and kafirs and lowest in the sorgos. Kernels of grain sorghum hybrids (RS 626, TE 77, and OK 612) grown in 1967 with nitrogen fertilization and irrigation had starch contents varying from 74 to 76%. Starch comprises 83% of the endosperm, 13.4% of germ, and 34.6% of the bran obtained by hand dissection of sorghum grain (Hubbard *et al.* 1950).

Amylose and Amylopectin Content.—Different types of starches are found in sorghums and other cereals. One kind of starch, amylose, is a polymer of glucose units united exclusively by α -1,4 linkages to give a linear chain (Fig. 4.1). It complexes with iodine to yield a blue color. Amylose dissolves with difficulty in water, from which it may be precipitated by butanol. The fraction of starch that is more soluble in water or an aqueous butanol solution is amylopectin. Amylopectin has, in addition to α -1,4 linkages, about 5% of α -1,6 bonds that give

a branched or bushy structure (Fig. 4.1). It gives a red color with iodine. Both of these starches have large numbers of anhydroglucose residues in their chains; amylose molecular weights range from 2 to 7×10^5 while amylopectin molecules are higher in molecular weight, 1 to 10×10^6 (Foster 1965).

Amylose may be determined in starch solutions either by amperometric titration with iodine or by photometric estimation of the blue



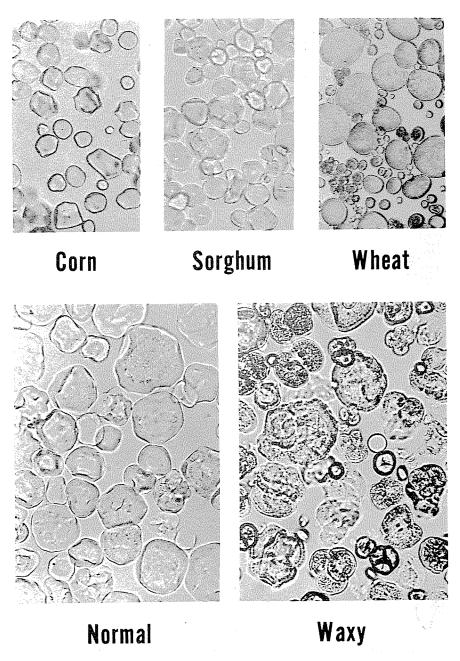


Amylopectin

Fig. 4.1. Molecular Structures of Amylose and Amylopectin

color formed with iodine. Deatherage et al. (1955) found that in normal varieties of grain sorghum, amylose content of starch ranged from 23 to 28%; amylopectin comprised the remaining starch. No sorghum grain was found to be high-amylose, above 28%, in contrast to corn where high-amylose strains have been developed. Starch in waxy sorghum varieties is essentially all amylopectin.

Starch Granule Characteristics and Gelatinization.—As seen in Fig. 4.2, starch granules from sorghum endosperm are in about the same size range as those from corn (6 to 24 μ diam), but on the average, starch granules of sorghum are slightly larger, 15 μ , as opposed to 10 μ for corn (Schoch and Maywald 1956). Granules in sorghum pericarp are much smaller. The starch granules in sorghum horny endosperm are



From MacMasters et al. (1957)

Fig. 4.2. Starch Granules: (Upper) Starch Granules of Corn, Sorghum, and Wheat; (Lower) Gelatinized Starch Granules of Normal and Waxy Sorghum

polyhedral and packed in close order, whereas in the floury endosperm they are round and more randomly spaced (MacMasters *et al.* 1957). The densities of sorghum starches are about 1.5 gm per ml (Barham *et al.* 1946).

On heating in water starch granules undergo gelatinization or disruption of their internal organization; they lose their birefringence, absorb water, and swell (Fig. 4.2). Gelatinization temperatures of sorghum starches extend from 68° to 76°C from initiation to complete gelatinization. On the other hand, gelatinization of corn starch occurs at 62° to 72°C (Leach 1965). Gelatinization temperatures are related to granule characteristics, such as average diameter, density, and amount of adsorbed substances, but are only slightly affected by amylose content.

Breakdown of starch by enzymes is more rapid when the granule is gelatinized. Novellie and Schutte (1961) used rate of amylolysis of sorghum starch as a means of estimating degree of gelatinization. Sodium chloride decreases the rate of gelatinization. Isolated sorghum starch gelatinizes more readily than does that in the grain.

A lower gelatinization temperature of sorghum starch would be desirable. Heusdens and King (1962) in studies of 125 sorghum introductions found several nonwaxy and waxy selections in which the starch had lower gelatinization temperatures. Those for some kaoliang varieties ranged from 63° to 71°C. Heusdens and King (1963) also found that gelatinization temperatures of grain sorghum starch increased 1° to 3°C when seed was stored over a period of 1 yr. Also, climatic conditions in the field influence gelatinization temperature.

When heated in water, waxy starch granules swell more rapidly than the nonwaxy (Fig. 4.2). This difference in granule behavior is due to the absence of the linear starch fraction in waxy sorghum. Swelling power is measured by the weight of sedimenting wet gelatinized starch obtained when starch is heated in water under prescribed conditions. Swelling powers of ordinary corn, sorghum, and rice starches are 24, 22, and 21; whereas that of waxy corn, sorghum, and rice starches are 63, 49, and 46, respectively, at 95°C (Leach 1965).

Phytoglycogen.—Watson and Hirata (1960) determined the starch content in sugary milo, feterita, and other sorghum grains (Table 4.6). In the sugary milo variety, the content of starch was only 31.5%. A water-soluble carbohydrate constituting 28.4% of the grain was extracted from it with 10% trichloroacetic acid. This polysaccharide resembled phytoglycogen from sweet corn. Phytoglycogen contains α -glucose residues, but is more highly branched than amylopectin and of lower molecular weight.

Table 4.6 carbohydrates in grain sorghums

		Grams per 100 Gm Grain, Dry Basis										
Variety	Genotype	Starch	Water-soluble Polysaecharide	Sugar	Fructose	Glucose	Sucrose	Maltose	Raffinose			
Combine kafir 54T	Wx Su	69.9		1.15	0.05	0.04	0.84	0	0.13			
Waxy kafir	wx Su	69.3	graduative.	1.39	0.14	0.14	0.95	0	0.12			
Waxy kafir, white	wx Su	68.6		1.07	0.09	0.09	0.77	θ	0.09			
Sugary feterita	Wx su	56.7	7.9	2.96	0.38	0.34	1.97	0.05	0.13			
Sugary milo	Wx su	31.5	28.4	2.68	0.05	0.22	2.2	0.02	0.1			

Source: After Watson and Hirata (1960).

Sugars in Sorghum Grain

Sugars translocated from the green parts of the plant are the precursors of the starch deposited in the grain. Kersting et al. (1961) followed the change in sugar and starch content that occurs during grain development. Sugar content rose until 12 days after pollination to a level of about 10% of the grain. It then dropped until at 20 days it reached 1%. However, the amount of sugar per kernel remained fairly constant (0.25 mg) until maturity. Concurrently, the starch content of the grain rose sharply after 5 days to a level exceeding 60%. After 35 days the starch and sugar content dropped slightly; this drop indicated a continuation of physiological activity after maturity.

A sugary mutant was reported by Karper and Quinby (1963) to have higher levels of sugars at all stages of development. At the milk dough stage it had 30% sugars versus 15% for the normal, and at the mature stage, 3.9 and 1.9% for sugary and normal, respectively.

The sugar content of mature sorghum grains ranges from 0.9 to 2.0% in normal varieties (Edwards and Curtis 1943). Using paper chromatography and chemical derivatives, Nordin (1959) identified in grain sorghum extracts the trisaccharide—raffinose—and the tetrasaccharide—stachyose—in addition to sucrose, fructose, and glucose. Watson and Hirata (1960) determined the amounts of sugars in several sorghum grains, including waxy and sugary types (Table 4.6). Sucrose, the major sugar in all varieties, is also the sugar that increases most in sugary grains. Sugary feterita had a higher content of fructose than any of the other varieties. Raffinose content was about the same in all grains.

Nordin (1959) followed changes in sugars during germination of sorghum grain. Sucrose concentration initially declined at 36 hr but then increased. Glucose and oligosaccharides showed increases only after 36 hr.

Hemicelluloses in Grain

In the whole sorghum grain, pentosans account for 2 to 3% of dry weight in a number of varieties analyzed by Edwards and Curtis (1943). Almost all the pentosans present are in the pericarp or bran. In this respect, sorghum resembles corn.

PROTEINS IN SORGHUM GRAIN AND PLANT

In sorghum there are a variety of proteins that exhibit different physical properties, biological activities, and nutritional values. Although

only 20 different amino acids are common constituents of plant proteins, they may be joined in different proportions and sequences to form large protein chains. The shape, solubility, digestibility, and nutritive value of protein molecules depend on their amino acid compositions and arrangements. Since many amino acids cannot be synthesized by humans, nonruminant livestock, or poultry, they are essential constituents of life-sustaining diets. The advent of automatic amino acid analyzers for analysis of acid hydrolyzates of protein has facilitated a rapid expansion of information on sorghum proteins.

Types of Proteins in Grain

On the basis of his observation that several different solvents used in sequence are required to remove almost all the protein of cereal grains, including sorghum, Osborne (1924) classified these proteins into: (1) albumins, soluble in water; (2) globulins, soluble in solutions of salts; (3) prolamines, soluble in solutions of ethyl alcohol; and (4) glutelins, soluble in dilute alkali. The greatest part of sorghum proteins are not extracted with water or salt solutions.

Albumins and Globulins.—Although small in quantity, the albumin and globulin fractions of sorghum proteins include enzymes and other biologically active substances. Jones and Csonka (1930) extracted defatted meals of 3 varieties of sorghum (a kafir, a milo, and a feterita) with 10% sodium chloride solution. They recovered only 12.7 to 13.3% of the total nitrogen of the meals in the extracts. Virupaksha and Sastry (1968) subjected endosperm meals from several sorghum varieties to extraction with water and then 1% sodium chloride solution. From 2 to 8% of the proteins from the different grains was removed by water while an additional 2 to 10% was extracted with salt solution (Table 4.7). Albumins and globulins can be precipitated from solution with 50% ammonium sulfate to yield fairly pure protein fractions.

Table 4.7 solubility fractionation of protein of grain sorghum endosperm

	Protein Content		% of $%$	Protein	
Variety	(Endosperm), $\%$	Albumin	Globulin	Prolamine	Glutelin
M-35-1 Siruguppa BS-81-3 Annigeri CSH-1 Bijapur 160 Cernum 361 Dochna	9.94 10.56 18.13 17.06 19.0	5.6 5.4 7.7 5.2 1.3	7.3 7.3 6.4 9.3 2.0	32.6 56.2 43.1 44.5 58.8	37.4 34.6 26.8 34.6 19.0

Source: Virupaksha and Sastry (1968).

Albumin and globulin fractions each consist of many different proteins. Sastry and Virupaksha (1967) used polyacrylamide gel disc electrophoresis to separate the proteins in both water and saline extracts of sorghum. Under the influence of an applied voltage, the various charged protein molecules migrated at different rates through the gels in buffered solutions. Several distinct bands of proteins stainable with dyes were detected in the gel after electrophoresis of the water or 1% sodium chloride extracts in pH 4.6 alanine-acetic acid buffers.

The amino acid composition of the globulin fraction of sorghum endosperm proteins determined by Virupaksha and Sastry (1968) is given in Table 4.8. Levels of lysine, threonine, arginine, methionine,

Table 4.8

Amino acid composition of protein fractions of a sorghum grain (csh-1 bijapur)

(Percent of Protein)

		Protein Fraction					
Amino Acid	Endosperm Meal	Globulin	Prolamine (Kafirin)	Glutelin			
Lysine	1.7	3.36	0.14	3.12			
Histidine	2.16	1.45	0.67	3.12			
Arginine	3.25	6.14	0.66	5.91			
Aspartic acid	6.25	8.68	6.72	9.07			
Threonine	3.81	4.87	_	4.88			
Serine	4.5	5.55	3.32	5.38			
Glutamic acid	29.75	15.8	25.07	24.08			
Proline	10.31	5.33	11.63	14.86			
Glycine	3.27	6.25	1.28	5.33			
Alanine	12.58	6.74	13.96	9.4			
Half cystine	1.08	1.99	Trace	1.21			
Valine	7.25	6.46	5.88	5.5			
Methionine	1.51	2.24	1.33				
Isoleucine	4.91	3.45	5.04	4.07			
Leucine	16.58	6.72	15.33	12.49			
Tyrosine	4.64	4.01	5.17	3.23			
Phenylalanine	6.4	4.77	5.84	4.9			

Source: Virupaksha and Sastry (1968).

and aspartic acid are much higher in the globulin fraction of sorghum proteins than in the total protein of the endosperm.

Kafirin.—The predominant proteins in the grain are prolamines or alcohol-soluble proteins. Unlike corn from which zein is readily extracted with 70% ethanol, little protein is extracted from sorghum meal with that solvent at room temperature. However, solutions of boiling 70% ethanol yielded 67% of the total protein of the kafir meal in experiments by Johns and Brewster (1916). Protein was precipitated from this solution by addition of sodium chloride and was designated kafirin. To avoid coagulation or denaturation of the protein, later

workers maintained the extraction at 60°C (Jones and Csonka 1930). In a study of 6 varieties, Virupaksha and Sastry (1968) found that protein soluble in hot 60% ethanol accounted for 30 to 60% of the grain protein (Table 4.7). The kafirin fraction is composed of at least 7 different components as indicated by bands after electrophoresis on polyacrylamide gels in pH 3.1 aluminum lactate buffer (Sastry and Virupaksha 1967).

Like zein from corn, kafirin is poor in nutritional quality, since it is deficient in several essential amino acids as shown in Table 4.8 from data by Virupaksha and Sastry (1968). The amount of lysine, arginine, histidine, glycine, and methionine is low in kafirins. Glutamic acid content is high, but it probably occurs primarily in the proteins as its amide glutamine, as indicated by a large amount of ammonia in acid hydrolyzates. The nonpolar amino acids—leucine, proline, and alanine—are also prominent components of kafirin. The solubility of kafirin in organic solvents, such as 60% ethanol, is due to its high content of these nonpolar amino acids and its low content of charged amino acids, such as lysine. Kafirin is low in tryptophan.

Glutelin.—Glutelin is the second major protein fraction. After removing saline and alcohol-soluble proteins, the meal is stirred with 0.4% sodium hydroxide for 2 hr at room temperature to extract the glutelin. From 20 to 40% of the meal protein, depending on the variety of sorghum (Table 4.7) was isolated in this manner (Virupaksha and Sastry 1968). The insolubility of glutelins in neutral solvents has been attributed to their high molecular weights caused by disulfide bonds in the amino acid cystine, which chemically link different protein chains. These bonds are labile to alkali. The glutelin of one variety of sorghum (CSH-1 Bijapur) has been analyzed for amino acid content by Virupaksha and Sastry (1968) who report that it has a higher content of lysine, histidine, arginine, and glycine than the kafirin (Table 4.8).

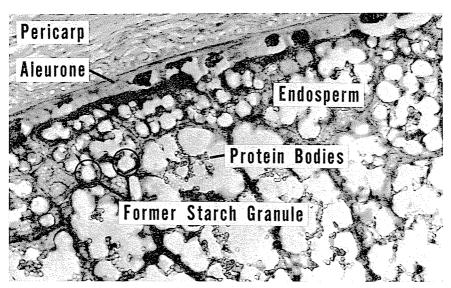
Since lysine, threonine, and methionine are essential amino acids most deficient in cereals, it is evident from Table 4.8 that the albumin and globulin proteins are the best in nutritional value; kafirin is the poorest, and glutelin is intermediate. The large amount of kafirin is responsible for the low nutritional value of sorghum grain protein. In comparing the protein distribution in grains of different varieties varying in protein amount (Table 4.8), Virupaksha and Sastry (1968) observed that a high content of kafirin generally accompanied high levels of total proteins. Although the protein level was enhanced, the protein nutritional quality was diminished. However, in the variety 160 Cernum, the increase in protein content was also accompanied by

a greater increase in the proportion of albumins, globulins, and glutelins than kafirin.

Development and Distribution of Proteins in Grain

In Table 4.4 it is seen that the different parts of the grain (germ, endosperm, and bran) differ in amount of protein. The grain parts also differ in their proportion of the different types of proteins. The prolamines are practically absent from the germ and hull, whereas they predominate in the endosperm. Consequently, germ proteins are higher in nutritive value than those of endosperm. The essential amino acids—lysine, threonine, methionine, and cystine—are present in the germ protein at levels of 4.1, 3.4, 1.5, and 1.0%; whereas in endosperm protein they account for only 1.1, 2.8, 1.0, and 0.8%, respectively (Pickett 1967).

The aleurone layer of endosperm is rich in albumins and globulins. Just inside the aleurone at the periphery of the horny endosperm is a dense layer of cells rich in kafirin (Watson et al. 1955). The larger amount of protein in the peripheral region of the endosperm is evident in Fig. 4.3 (Wall 1967). This illustration shows a photomicrograph of a section of sorghum grain from which the starch was removed



From Wolf and Khoo (in Wall 1967)

Fig. 4.3. Section of Sorghum Grain Showing Protein Matrix and Protein Bodies. Starch Removal by Treatment with α -Amylase

by amylase treatment. It reveals a network of proteins in which small subcellular bodies are located. These bodies are presumed to be sites of storage of kafirin. The matrix protein in which the protein bodies and starch are embedded may consist of glutelin. The insolubility of most of the endosperm protein and the manner in which this protein binds the starch granules contributes to the difficulty of digesting sorghum grain. Also, this cellular organization must be disrupted to facilitate starch-protein separation by wet-milling.

As the grain progresses from the milky to the mature stage, there is a rapid increase in the amount of alcohol- and alkali-extractable proteins. In contrast, the albumin and globulin proteins, which in the early stages of seed development predominate, constitute a smaller fraction of the total protein of the mature seed (Taira 1964). As the seed ripens, alanine, isoleucine, leucine, glutamic acid, phenylalanine, and proline increase while lysine, glycine, aspartic acid, and arginine decrease.

Effect of Agronomic Factors and Variety on Protein

Fertilizer use and location of planting not only affects protein level, but also influences the amino acid composition of the protein. Waggle et al. (1967) found that nitrogen fertilization linearly increased the proportion of glutamic acid, proline, alanine, isoleucine, leucine, and phenylalanine in the protein while that of lysine, histidine, arginine, threonine, and glycine was reduced. Nitrogen fertilization evidently favors increased deposition of kafirin in the grain. Variations in protein content due to location also were shown to influence the amino acid content of the grain.

A major factor that determines the amino acid composition of the protein of sorghum grain is variety and hybrid. Analysis of 15 different hybrids grown at two locations by Deyoe and Shellenberger (1965) showed variations in protein content, and demonstrated significant differences in amino acid composition of the protein. Some of their results are given in Table 4.9. The correlations of lysine, arginine, and glycine to amount of protein were negative. (Waggle and Deyoe 1966). Apparently in hybrids developed in the United States, increases in protein content have so far resulted in higher levels of protein deficient in certain essential amino acids.

Virupaksha and Sastry (1968) examined sorghum grains of 44 varieties from the World Collection and of 5 hybrids for protein and for lysine. Protein values ranged from 8.5 to 18.2%. In general, they also established a correlation between high-protein content and a low proportion

TABLE 4.9 COMPARISON OF AMINO ACID ANALYSES OF SORGHUM GRAINS, OTHER CEREALS, AND SOYBEAN MEAL WITH FAO PROTEIN PATTERN (Percent of Protein)

		So	rghums						FAO
Amino Acid	RS 6101	59-MH1	CSH-1 ²	160 Cernum ²	$\mathrm{Corn^3}$	Wheat ³	${ m Rice^4}$	Soybean Meal ⁵	Provisional Pattern ⁶
Lysine	2.1	1.8	1.7	3.1	2.7	2.5	3.4	6.9	4.3
Histidine	2.2	2.0	2.0	2.3	3.03	2.0	2.2	2.6	
Arginine	2.8	2.5	2.9	4.9	5,2	3.6	2.1	8.4	
Aspartic acid	6.6	6.0	6.2	9.1	6.8	3.4		12.6	
Threonine	3.2	3.0	3.2	3.6	3.6	2.5	3.4	4.3	3.3
Leucine	4.4	4.2	4.7	4.7	5.2	4.7		5.6	
Glutamic acid	21.5	21.9	23.6	23,4	21.3	29.3		21.0	
Proline	8.1	7.7	8.6	12.7	10.0	10.3		6.0	
Glycine	3.3	2.9	2.8	3,2	4.0	3.4		4.5	
Alanine	9.5	9.5	13.5	10.3	8.1	3.0		4.5	
Half cystine	1.1	1.0	Accession	*********	1.6	4.0	1,2	1.6	1.7
Valine	5.2	4.7	5.4	4.2	4.7	2.9	6.2	5.4	2.8
Methionine	1.5	1.5	0.83	1.1	1.7	1.0	1.4	1.7	1.7
Isoleucine	3.9	3.8	3.7	3,8	3.5	4.2	5.2	5.1	4.3
Leucine	13.3	13.9	13.2	12.7	12.4	6,6	8.2	7.7	4.9
Tyrosine	1.6	1.4	1,9	2.2	4.4	3.5	5.7	3.9	2.5
Phenylalanine	5.0	4.8	5.1	4.6	5.0	4.9	5.2	5.0	2.9
Tryptophan	1.0	1.0			1.0			1.3	1.1
% Protein	10.0	10.1	16.5	17.7	10.0	12.0	9.0	61.4	

¹ Deyoe and Shellenberger (1965).

² Virupaksha and Sastry (1968).

³ Unpublished data by authors.

⁴ National Academy of Sciences (1958).

 ⁵ Rackis et al. (1961).
 ⁶ FAO/WHO (1965).

of lysine in the protein. However, a marked exception from this trend was exhibited by the high-protein variety 160 Cernum (Table 4.9). In corn, high-lysine varieties have been discovered that have a changed protein composition, less zein, and more of the other components (Mertz et al. 1964). This observation has encouraged exploration for highlysine sorghum varieties.

Nutritional Value of Grain Protein

How does sorghum grain compare with other cereal grains and plant seeds in amino acid composition? Table 4.9 summarizes the amino acid content of the protein in several cereals and soybean meal, and compares their analysis to the FAO recommendations for essential amino acid composition of a balanced protein in human diets. The protein of hybrid U.S. sorghum grain is low in lysine, threonine, methionine, and tyrosine. It is also low in arginine, histidine, and glycine, which are essential for some animals. The tryptophan level is near the minimal requirement but is higher than that of corn.

Cereals are generally supplemented with a suitable protein source, such as defatted and properly toasted soybean meal (Table 4.9), to provide the level of protein required in formulations for optimum growth of young nonruminants and for nutrition of preschool children. For example, soybean meal is often added to sorghum rations to provide an adequate level of lysine and threonine, but methionine then becomes the limiting amino acid in the formulation.

Proteins in Stem and Leaf

Because the vegetative part of the sorghum plant is primarily used as fodder for ruminants, less attention has been given to its protein components. Klimenko and Goldenberg (1960) extracted proteins from the stems and leaves of several hybrids and varieties of sorghum with 7% sodium chloride and then with 0.2~M sodium hydroxide. From 35 to 45% of the nitrogen was extracted with these reagents. From 67 to 76% of this protein was soluble in the saline solution; the rest was extracted by the alkali. About 2% of the extracted nitrogen was nonprotein nitrogen. Amino acid analysis of leaf proteins from various cereals indicate they have suitable levels of all essential amino acids.

LIPIDS

The lipids in sorghum are important to animal and human nutrition but may contribute to the development of off-flavors and rancidity in sorghum-based food products. Two general types of solvents are used to extract lipid material from sorghum. Nonpolar solvents, such as hexane, extract principally triglycerides with lesser amounts of hydrocarbons, sterol esters, fatty acids, monoglycerides, diglycerides, and sterols. This mixture is usually termed crude fat or oil. The more polar solvents, such as *n*-butyl alcohol or chloroform-methanol, extract fatty acids, phospholipids, glycolipids, and lipoproteins. Newer methods of analysis, thin-layer and gas-liquid chromatography, make it possible to separate rapidly and identify components of these complex crude lipid mixtures.

Grain fat or oils normally contain relatively low concentrations of free fatty acids. The major portion of fatty acids are combined in mono-, di-, and triglycerides and in phospholipids. For ease of analysis, these fatty acids are liberated from parent compounds and converted to more volatile methyl esters. The fatty acid methyl esters may then be separated by distillation or by gas-liquid chromatography. Chain length and degree of unsaturation of fatty acids determine the physical and chemical properties of the oils. The extent of unsaturation (iodine number) affects the nutritional value and storage stability of the oil.

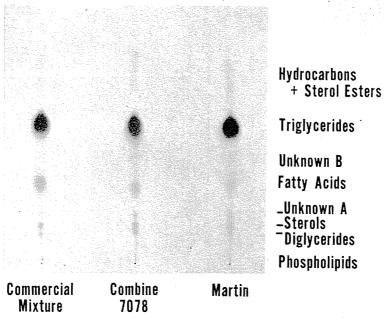
Grain Lipids

The distribution of nonpolar lipids in five varieties of sorghum grain and their hand-dissected fractions closely resembles that of corn (Hubbard *et al.* 1950). Average oil content of the whole grain is 3.6%, with oil contents of the endosperm, germ, and bran, 0.6, 28.1, and 4.9%, respectively. The endosperm contains 13% of the total oil in the kernel; the germ, 76%; and the bran, 11%. The petroleum ether extract from sorghum bran consists mostly of wax rather than oil. Compositions are similar among the five varieties.

Nonpolar Lipids.—An examination of nonpolar lipids from ground whole sorghum grains of three varieties by thin-layer chromatography indicated the presence of a number of different classes of substances (Wall 1967) (Fig. 4.4). The largest single fraction was composed of triglycerides. Smaller amounts of hydrocarbons, sterol esters, fatty acids, monoglycerides, diglycerides, sterols, and phospholipids were also present. The triglycerides were further separated by gas-liquid chromatography. The 3 sorghums had similar triglyceride compositions averaging as: C_{50} , 3%; C_{52} , 32%; C_{54} , 64%; and C_{56} , 1%. Subscripts refer to the total number of carbon atoms in the three fatty acids esterified to glycerol in the triglycerides.

Several investigators have determined the physical properties and





From Blessin (in Wall 1967)

Fig. 4.4. Thin-Layer Chromatographic Separations of Sorghum Nonpolar Lipids from Three Different Sorghum Grains

composition of sorghum grain oil (Table 4.10). Kummerow (1946A, B) analyzed oil after extracting ground, wax-free, whole grain, whereas Bertoni and his coworkers (1963) examined only germ oil. The origin of the oil investigated by Denisenko and Volkova (1960) and Durio $et\ al.\ (1965)$ was not specified. Sorghum grain oil was slightly less saturated than corn oil and contained more oleic and stearic and less linoleic, myristic, and hexadecenoic acid than corn oil (Kummerow 1946A, B). Kummerow (1946A, B) also reported that neither corn nor sorghum grain oil contained linolenic acid or fatty acids above C_{18} , whereas Denisenko and Volkova (1960) and Bertoni $et\ al.\ (1963)$ found that sorghum grain oil had from 1 to 2% linolenic acid.

Baldwin and Sniegowski (1951) studied the fatty acid composition of lipids associated with four main fractions from wet milling of commercial hybrid sorghum. These fractions included germ (52% fat), starch (1% fat), gluten (7% fat), and fiber (3% fat). Fatty acids were analyzed by fractional distillation and spectroscopic tech-

4.7

39.5

46.5

TABLE 4.10 PROPERTIES AND COMPOSITION OF SORGHUM GRAIN OIL

5.8

36.2

49.4

42.48

1.15

Durio et al.

(1965)

0.2

0.4 13.2

1.3

2.0

30.5

49.7

2.0

Property or Component

Refractive index (25°C)

Saponification value

Thiocyanogen value

Unsaponifiable matter (%)

Neutralization equivalent

Laurie acid (%)
Myristic acid (%)
Palmitic acid (%)
Hexadecenoic acid (%)

Color

Acid value

Iodine value

Acetyl value

Stearic acid (%)

Oleic acid (%)
Linoleic acid (%)

Linolenic acid (%)

25.6

52.8

1.9

niques. Gluten and germ fats had approximately similar amounts of oleic and linoleic acids, but more polyunsaturated fatty acids were present in the gluten. Gluten and fiber fats contained up to 10% unsaponifiables and about 20% free fatty acids.

The lipids associated with starch influence paste clarity and starch insolubility. The fat content of sorghum starch by methanol extraction is 0.32%, and by acid hydrolysis, 0.72% (Lindemann 1951). Starch fat is 90% free fatty acids. It contains less of the unsaturated acids, oleic and linoleic, with correspondingly higher amounts of saturated acids, primarily palmitic, than does germ, gluten, and fiber lipids. The composition of fats in sorghum starch is similar to that in corn starch.

Wax.—Sorghum grain contains approximately 0.25% wax (Kummerow 1946A). The wax content of sorghum grain is 50 times more than that of corn. The wax is easily removed by extracting the unground grain with hot hexane. The characteristics of waxes from four varieties of sorghum grain were compared by Bunger and Kummerow (1951) with those of carnauba wax for possible industrial use. Sorghum grain wax has an acetyl value of 7; acid number, 13; iodine number, 18; and saponification number, 30.

The composition of sorghum grain wax was studied by fractionating it on columns of tricalcium phosphate and silicic acid (Dalton and Mitchell 1959). Of the material recovered from the columns, approximately 5% was paraffins, 49% esters of long chain fatty alcohols, and 46% free alcohols. Melting points, X-ray diffraction studies, and infrared absorption spectra indicated that each of these fractions was a mixture of related substances rather than a single compound. Chain lengths of the individual paraffin hydrocarbons, alcohols, and fatty acids were mostly 26, 28, and 30 carbons.

Phospholipids.—Major components of the bound lipid fraction extracted from sorghum grain with methanol-chloroform are phospholipids. These substances represent about 5% of the total lipids. Boissy and Perles (1965) fractionated half of the phospholipid into a 95% ethanol-soluble lecithin fraction; the remainder constituted the cephalin portion. The lecithins consist of glycerol, fatty acids, phosphorus, and choline associated by ester linkages. Thin-layer chromatography of the cephalin fraction revealed the presence of phosphatidylethanolamine and phosphatidylserine, compounds which resemble lecithin except that choline is replaced by ethanolamine or serine. An inositol phosphatide was also detected on the chromatogram. This compound contained phosphorus, glycerol, inositol, and fatty acids in the proportion 1:1:1:2.

Leaf and Stem Lipids

Lipids in the leaf and stem of sorghum have received limited attention despite their importance in forage. Fractionation of the lipids of hexane and acetone extracts of sorghum leaf and stem by countercurrent distribution yielded five distinct components (Burnett et al. 1958). The amounts of phosphorus in various fractions were 0.04 to 1.20%; of nitrogen, 0.08 to 1.23%. Fatty acid composition of the fractions was determined by gas-liquid chromatography following their conversion to methyl esters (Burnett and Lohmar 1959). The major unsaturated acid was linolenic, which was concentrated in the more polar fraction. The major saturated acid, palmitic, was relatively evenly distributed throughout the fractions. The chief acid of the leaf and stem was linolenic. Sorghum leaves and stems are lower in linoleic acid and much higher in saturated acids than other leaf fats. Apparently the fatty acids of sorghum leaf and stem are present largely in phospholipids, not triglycerides.

Plant waxes were compared in forage (Atlas) and grain (Western Blackhull) sorghums at different stages in plant growth by Cannon and Kummerow (1957). Plant waxes are produced throughout the growth period by both types of sorghums. A constant level of wax is reached at heading. At maturity, leaves of both varieties contain about 0.30% wax but the stalk of the grain sorghum contains 0.60%, while that of the forage type contains less than 0.33%. The waxes of the mature leaf, stalk, and grain vary in chemical composition. Corn leaves contain only ½ the wax present in sorghum leaves and corn stalks only ¼ of the wax as those from sorghum.

PHENOLICS

Phenolic compounds contribute flavor and color to sorghum-derived feeds and foods. In addition, they may interfere with digestibility and can be toxic, especially in forage sorghums. The phenolic category consists of many aromatic organic compounds including flavonoids, cyanogenic glycosides, tannins, and lignin. The structures of 4 typical phenolic compounds are shown in Fig. 4.5. The flavonoid group encompasses the anthocyanidins, anthocyanins, anthocyanogens, flavanones, and other related 15-carbon substances. Numerous plant phenolics—illustrated by dhurrin and the flavonoid glycosides, such as cyanin—occur as chemical combinations of an aglycone with various sugars.

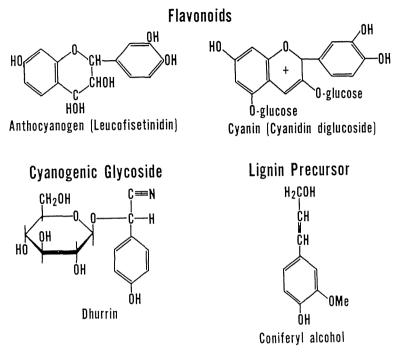


Fig. 4.5. Structures of Some Typical Phenolic Compounds

Grain Phenolics

Pigments.—Varieties of sorghum differ greatly in seed color, ranging from white to dark brown, depending on the presence of phenolic pigments. These colors may be transferred to the grits during dry milling and to the starch and gluten during wet milling. Phenolic pigments may cause bitterness and unpalatability of the grain and its products. Bird-proof varieties of grain generally have high levels of phenolics. Grain types with less pigment are being developed for use in some areas of the United States.

Pigments in several red varieties of sorghum grain were investigated by Nip and Burns (1968). Orange pigmentation occurred in epicarp, in cross-cell and tube-cell layers of the pericarp, and in seed-tip portions of the grain. Anthocyanin, flavone, and aurone-type compounds were tentatively identified by chromatographic separation and characterization, spectrophotometric measurements, color reactions, and hydrolysis products.

The red-brown pigments of the seeds of a sweet sorghum were extracted with methanol and separated on calcium carbonate columns

by Herman *et al.* (1958). Two anthocyanidins, blood red sanguisorghuidine and ruby red rubisorghuidine, were isolated. The pigments are pH-sensitive and soluble in alcohols and acetone, but are insoluble in water and nonpolar solvents.

A group of colorless flavonoid pigment precursors, termed leucoanthocyanins or anthocyanogens, may be responsible for the development of pigments during processing of sorghum grain. These substances become colored in acid solutions. The anthocyanogens apparently impart astringency to foods and beverages. Anthocyanogens were detected in yellow milo and red kafir sorghums, but not in white kafir, waxy, and yellow endosperm varieties (Blessin et al. 1963). When present, these compounds are located mainly in the pericarp and are generally absent from the endosperm. The anthocyanogens in aqueous extracts of the whole grain were purified on ion-exchange resins and separated by paper chromatography. Based on spectral absorption, fisentinidin was tentatively identified as one of the reaction products resulting from treatment of the anthocyanogens with concentrated hydrochloric acid at room temperature.

Further investigation indicated the presence of three anthocyanogen-like flavonoids—chromagens I, II, III—in methanol extracts of sorghum pericarp (Yasumatsu *et al.* 1965). The compounds are polymerized substances similar to those reported in lignin. Upon hydrolysis the three chromagens yielded a flavanone, probably eridictyol, and an anthocyanidin, pelargonidin.

Tannins.—Sorghum grain varieties with brown seed color are characteristically high in tannin. High tannin levels are thought to affect their palatability in feed rations and may be responsible for their mold resistance. Diets containing sorghum grain high in tannin retarded growth in poultry similar to equivalent levels of tannic acid (Chang and Fuller 1964). Tannins were extracted with hot water and determined colorimetrically with Folin-Denis reagent. Tannin contents in the brown-seeded sorghums ranged from 1.3 to 2%, compared to a range of 0.2 to 0.4% in other common varieties. Barham et al. (1946) found that sorghum tannins did not react with some reagents that yielded colors with tannic acid. They concluded that sorghum tannins may consist of condensed flavonoids, whereas tannic acid is a gallic acid derivative.

Leaf and Stem Phenolics

Cyanogenic Glycosides.—The hydrocyanic acid or prussic acid (HCN) produced in forage sorghum is of special concern to cattle feeders since certain levels of HCN are toxic. Although the HCN content of a plant

is often reported, growing sorghums do not contain appreciable free HCN. Dunstan and Henry (1902) in search of the poisonous substance in sorghum, isolated dhurrin. HCN is liberated from dhurrin by the action of enzymes.

An improved isolation method for dhurrin from extracts of sorghum leaves has been reported (Mao et al. 1965) which includes the following steps: (1) Removal of sugars by yeast fermentation, (2) deionization with ion-exchange resins, and (3) cellulose column chromatography. Dhurrin has a melting point of 163° to 165° C; $[\alpha]_{\rm D}^{28}$ —64° in water, —65° in ethanol; and pKa 8.93. A paper chromatographic study of sorghum extracts indicated that dhurrin is the only cyanogenic glycoside present. Physical and chemical data, including ultraviolet and infrared spectroscopy and nuclear magnetic resonance, established that dhurrin is p-hydroxy-L-mandelonitrile- β -D-glucopyranoside (Fig. 4.5).

Evidence has been presented by Gander (1966) for the presence of a phenolic substance not previously reported in sorghum seedlings. The substance is similar to p-hydroxymandelonitrile- β -glucoside in that it is derived from p-glucose and L-tyrosine.

The amounts and distribution of dhurrin were determined in germinating etiolated sorghum seedlings (Akazawa et al. 1960). Upon homogenization of the tissues, the cyanogenic glycoside that occurs in these plants is enzymically hydrolyzed to form equimolar amounts of HCN and p-hydroxybenzaldehyde. The glycoside is localized in aerial shoots of the plant. Seeds of sorghum do not contain the glycoside. HCN in sorghum plants at various stages of growth ranges from traces to 335 mg per 100 gm. Young plants under 3 weeks have more HCN than do mature plants; generally, leaves contain more than stems. contain more HCN than the principal stem since they are usually less developed. HCN content varies considerably with variety. (1953) reported that nitrogen fertilizer had no influence on HCN content, but Nelson (1953) stated that it increased the HCN level. Braithwaite (1952) found that the concentration of HCN was greater in young plants grown under drought conditions than in those grown under more favorable moisture conditions (Heinrichs and Anderson Strains can be cut safely only after 50% of their total projected growth has taken place. Curing decreases HCN in sorghum forage.

Anthocyanins.—Wheatland sorghum seedlings show a marked reddening of stems at early stages of growth because of anthocyanin formation. Prolonged irradiation at moderately high intensities is required for anthocyanin synthesis. Anthocyanin synthesis in seedlings of

sorghum is controlled by two photoreactions (Downs and Siegelman 1963). Four-day-old internodes of sorghum grown in complete darkness contained little or no detectable flavonoids, although $C_{\rm 0}$ phenolic compounds and dhurrin were relatively abundant (Stafford 1965). After light treatment, flavonoids were identified in the nongrowing portions of either intact or excised internodes (Stafford 1965). Two were anthocyanidins—a red acylated cyanidin-3-glucoside (apigenidin) and orange luteolinidin. Another was probably the flavone, luteolin. These compounds were identified by absorbance spectra and paper chromatography.

The sorghum kernel is encased in glume tissues, which varies considerably in color. Flavonoid pigments were determined in glume tissue from 19 different Nigerian sorghums (Stanton *et al.* 1959). Paper chromatograms revealed three spots: blue-purple, red, and brown. Black and dark mahogany glumes contained all three types of pigments. The pigments in sorghum glumes are not combined with sugars.

Lignin

Although closely associated with cellulose in fibrous tissues, lignin is a complex phenolic substance. In other species, lignin appears to be a crosslinked polymeric material formed from coniferyl alcohol (Fig. 4.5) and related substances. Analysis for lignin involves determining insoluble organic matter remaining after hydrolysis of protein and carbohydrate in tissues extracted with organic solvents.

Crude lignin constitutes approximately 17% of the glumes of Leoti sorghum (Edwards and Curtis 1943), and 10 to 20% of the leaves and stems of forage sorghum (Kuniak and Slavik 1960; Bettini and Proto 1960; Lengyel and Annus 1960; Sorgato 1949). Lignin content of forages increases with plant maturity, with values ranging from 3.23 to 6.72% for the 1st and 5th cutting, respectively (Achacoso et al. 1960). Lignin is higher in stems than leaves at all stages of growth. Application of up to 120 lb nitrogen per acre has no significant influence on lignin content. Grain sorghums and grass sorghums, such as sudangrass, are highest in lignin content; sweet sorghums are lowest (Table 4.5) (Lengyel and Annus 1960). The correlation between lignin and crude protein was negative, but positive between lignin, crude fiber, and dry matter.

NONPHENOLIC PIGMENTS

Chlorophylls are responsible for green pigmentation, and carotenoids cause yellow and red colors in natural products. Chlorophylls con-

tain a nitrogenous porphyrin nucleus, whereas carotenoids are composed solely of a number of condensed isoprene units. Carotenoid pigments include two general classes: the carotenes, and the xanthophylls, which are similar to carotenes but contain hydroxyl groups. The carotenes are important in feeds as vitamin A precursors and as a source of yellow color in milk and body fat of cattle. Most xanthophylls, for example zeaxanthin, do not exhibit vitamin A activity, but do impart the desirable yellow color to egg yolks and to the skin of broilers.

Carotenoids in Sorghum Grain

At present, corn is the only grain providing significant amounts of xanthophylls and carotenes in mixed feeds in the United States. However, sorghum varieties found in Nigeria and India with a yellow endosperm contain appreciable carotenoids. Plant breeders in the United States have developed yellow endosperm types which contain larger amounts of carotenoids. The grain of common varieties of sorghum contained about 1.5 ppm total carotenoids, while crosses obtained with yellow endosperm varieties contained as high as 10 ppm (Blessin *et al.* 1962). However, grain of common yellow corn hybrids have 21 ppm carotenoids.

Major carotenoids present in extracts of yellow endosperm sorghum crosses were identified after chromatographic separation. These compounds were zeaxanthin, 2.8 ppm; lutein, 2.2 ppm; and β -carotene, 0.8 ppm. Two other unidentified pigments were also present—xanthophyll I, 1.8 ppm; and xanthophyll II, 0.4 ppm.

A major problem in the development of yellow endosperm varieties is the rapid loss of carotenoids from sorghum grain in the field (Blessin et al. 1962). When exposed to weathering after pollination, sorghum retained only 50% of the carotenoids present in protected seed heads. Carotenes and xanthophylls decreased continuously with no preferential loss of individual carotenoids. Colored pericarps did not inhibit loss of carotenoids.

The inheritance of β -carotene content was studied in the grain of eight sorghum crosses and of their parents (Worzella *et al.* 1965). β -Carotene in the grain of the parents ranged from 0.22 to 3.23 mg per kg. Although concentration in the F_2 segregates generally fell between the parents, there was a preponderance of segregates with a β -carotene content lower than the midparent value. Positive correlation coefficients were obtained between endosperm color and β -carotene content in the F_2 .

Nonphenolic Pigments in Leaf and Stem

Various pigments account for 9% of the total solids extracted from sorghum leaf and stem with acetone and hexane (Burnett *et al.* 1958). On a dry weight basis two types of chlorophyll, a and b, were the major pigment components, 6,200 and 1,800 ppm. β -Carotene (100 ppm) and other carotenoids (40 ppm) were also present.

VITAMINS

The importance of vitamins in grain and forage to animal nutrition is well established. Vitamins generally are classed according to their solubility. Water-soluble vitamins include niacin, thiamine, folacin, riboflavin, B_{12} , and C. Fat-soluble vitamins include tocopherol (E) and the carotenes, which are vitamin A precursors.

Grain Vitamins

Data on sorghum grain vitamin composition averaged from a number of sources are given in Table 4.11 (Hubbard et al. 1950; Tanner et al. 1947; Naik and Abhyankar 1955). Compared to corn, sorghum grain contains approximately the same quantities of riboflavin, and pyridoxine, but more pantothenic acid, nicotinic acid, and biotin (Tanner et al. 1947). Grain sorghum compares favorably with wheat and rice with regard to levels of thiamine and niacin, but it is poorer in riboflavin (Naik and Abhyankar 1955).

The average distribution of vitamins in hand-dissected fractions from 5 varieties of sorghum (Table 4.11) was determined by Hubbard *et al.* (1950). The germ has 2 to 5 times the quantity of vitamins present in endosperm and bran. The germ and bran contain about equal amounts of riboflavin, whereas the bran and endosperm contain about the same concentration of niacin, pantothenic acid, and pyridoxine. The amount of individual vitamins varies considerably among varieties.

The development of sorghum varieties with high-niacin content is possible (Tanner *et al.* 1949). Niacin in grain from Westland plants (43.0 to 49.1 μg niacin per gm) by Cody cross (66.9 to 72.9 μg per gm) was determined. Some F₃ generations had as much as 124 μg of niacin per gram.

The biological availability of niacin in sorghum is important since pellagra in humans has been associated with sorghum diets. Niacin in sorghum grain estimated by chemical and microbiological methods before and after hydrolysis by acid or alkali was not significantly dif-

 ${\bf T}_{\rm ABLE} \ \ 4.11$ average vitamin composition of whole sorghum grain and fractions

				Vitamin Co	ontent, μg/Gm			
Fraction	Niacin	Pantothenic Acid	Riboflavin	Biotin	Pyridoxine	Thiamine	Ascorbic Acid (C)	Choline
Whole grain	45.3	10.4	1.3	0.20	4.7	3.3	21.0	420.0
Endosperm	43.7	8.7	0.9	0.11	4.0	-	Balance Tribate	***************************************
Germ	80.7	32.2	3.9	0.57	7.2		Account	
Bran	44.0	10.0	4.0	0.35	4.4			-

After Hubbard et al. (1950).

ferent (Belavady and Gopalan 1966). Therefore, pellagra cannot be explained on the basis of a primary niacin deficiency due to an unavailable form in sorghum. These results are in disagreement with findings on the availability of niacin in milo for swine (Luce et al. 1967). The addition of crystalline niacin improved weight gains of pigs fed rations containing sorghum grain as the primary source of energy. Total niacin of the grain sorghum ration equalled or exceeded the recommended level. These experiments indicate that niacin in sorghum grain is largely unavailable to swine.

Leaf and Stem Vitamins

Carotene content of forage material from sorghum and sudangrass has been reported as 6.0 and 2.0 μg per gm (Acha and Dora 1955). Tocopherol content in leaves decreased from 333 μg per gm after 1 week of growth to 150 after 5 weeks and then increased to 243 μg per gm of dry matter after 11 weeks (Ramanujan and Anantakrishnan 1958). The leaf-to-stem ratios were 2.9, 1.7, and 6.1 at 1, 5, and 11 weeks respectively. At corresponding times carotene values in leaves were 945, 592, and 617 μg per gm. The apex of the leaf contained more tocopherol and carotene than the base or lamina. Tocopherol was greater in plants grown in the winter than in plants grown in the summer. Shade drying of the plants did not preserve more tocopherol or carotene than sun drying.

OTHER ORGANIC SUBSTANCES IN SORGHUM

Acids

Titratable acidity in sorghum stem juices normally increases during the harvest season—often doubling or tripling before the season ends (Webster et al. 1954). This increase in acid compounds has been cited as a reason for poor quality syrup late in the season. The major organic acid of sorghum juice is aconitic acid, 12 mg per ml, which may settle out as the calcium salt during concentration of the neutralized juice. Mader and Webster (1954) using partition chromatography on silica gel columns, found that tartaric, malic, and citric acids were present in juices at 11, 5, and 2 mg per ml, respectively. Oxalic, fumaric, and acetic were in a lower concentration. They found large differences among varieties.

Disease resistance and detrimental effects of plant residues on crop growth have been attributed to phenolic acids in sorghum. Guenzi and McCalla (1966) quantitatively estimated five phenolic acids, ferulic,

p-coumaric, syringic, vanillic, and p-hydroxybenzoic, in sorghum plant residues. In alkaline and acid hydrolyzates, p-coumaric acid was present in the largest amount, 1.5%. Almost all the phenolic acids occur in the plant combined with sugars.

Phytic acids, the phosphoric acid esters of inositol, are widely distributed in plants, especially in seeds. Phytic acid is capable of forming complexes with certain ions. It has been implicated as impairing the calcium and zinc metabolism of animals. Phytic acid is determined by phosphorus analysis of the insoluble iron salt. Wang et al. (1959) established that the phytic acid in sorghum grain occurs primarily as the inositol hexaphosphate. These workers determined the phytic acid in various parts of several varieties of sorghum grain. Phytate phosphorus ranged from 0.20 to 0.37% in the whole grain. The germ was highest in phytate ranging from 0.54 to 1.91%; bran, from 0.19 to 0.49%; and grits, from 0.03 to 0.07%. Becker (1950) found that of various grains analyzed, sorghum had the highest percentage of its phosphorus present in phytic acid, 75%. Johri and Kehar (1962) report that green sorghum fodder contains 149.0 mg phytic acid phosphorus per 100 gm dry matter, which amount accounts for 33% of its total phosphorus. In sorghum straws, 65.5 mg of phytic acid phosphorus occurs per 100 gm dry matter. Sudangrass contains 292 mg phytic acid per 100 gm dry matter.

About 2% of the nitrogen of sorghum grain is contained in low-molecular-weight compounds. Reindel and Scheublein (1959), using 2-dimensional paper chromatography, identified 21 different amino acids in extracts of sorghum. Compounds included were ornithine, glutamine, asparagine, γ -aminobutyric acid, and α -aminobutyric acid, as well as other amino acids.

Growth Substances

Like corn and some other grains, sorghum grain appears to be a source of auxin-like growth substances related to indoleacetic acid. Netien (1965A) found that the growth of tissue cultures of artichoke parenchyma was proportional to the amount of sorghum extract added to the tissue culture. Auxin activity was contained mainly in basic and neutral fractions of grain extracts.

Gibberellins serve to stimulate germination and enzyme development in grains, as well as to promote cell elongation. Extracts of immature sorghum grain were concentrated and applied to germinating pea plants (Netien 1965B). The response in growth indicated that gibberellin-like material was present at a concentration of 125 $\mu \rm g$ per kg grain.

Nucleic Acids

The importance of deoxyribonucleic acid (DNA) as the agent governing the genetic character of plants and of ribonucleic acid (RNA), which determines the synthesis of proteins, has prompted their study in sorghum. The nucleic acids are large polymers consisting of chains of nucleotides whose sequences serve as a code for the arrangements of amino acids in proteins. Nilson and Pauli (1964) investigated the levels of RNA and DNA during root growth of sorghum seedlings. The content of both nucleic acids was higher in the roots of 2 hybrid sorghums, RS 610 and RS 501, than in 2 milo varieties. RNA and DNA concentration was greatest in root parts where cell division was continuing. Nirula et al. (1961) tried to correlate the size of chromosomes in sorghum cells with the nuclear DNA content. In sorghums differing in chromosome number, size, and stainability, the DNA content per unit length differed. They attributed this difference to variations in observed heterochromatin in the chromosomes of the different sorghums.

MINERALS

Levels of minerals in sorghum grain and plant parts depend on a number of variables, such as variety, soil conditions, temperature, rainfall, and fertilizer.

Grain Minerals

Although numerous studies have been made of the composition of minerals in sorghum grain, the most comprehensive data are reported by Pinta and Busson (1963). Phosphorus, magnesium, potassium, and silicon are the major minerals in sorghum grain with lesser amounts of calcium and sodium also present (Table 4.12). Data on other mineral elements ranging in concentration from less than 0.5 to 150 ppm are also given. About 20% of the total calcium and 13% of the total phosphorus are in the fibrous seed coat (Kurien et al. 1960). From 40 to 75% of the phosphorus is present as phytate phosphorus (Naik and Abhyankar 1955). Variations in calcium and phosphorus among some varieties and hybrids are given in Table 4.3 (Bressani and Rios 1962).

Leaf and Stem Minerals

Analyses for minerals in sorghum roughages have been compiled (National Academy of Sciences 1958). Average data in Table 4.13

Table 4.12 composition of minerals in sorghum grain

Element	Range	Average	
	Concentration, %		
Si	0.1-0.3	0.20	
Na	0.01 - 0.02	0.02	
K	0.35 - 0.52	0.40	
\overline{C} a	0.02-0.03	0.02	
$\overline{\mathrm{Mg}}$	0.13-0.23	0.18	
P	0.43-0.63	0.49	
Total ash	1.9 - 2.5	2,20	
	Concentration, Ppm		
Al	5-69	17.6	
B	1-3	1.3	
Ba	0.2-2	0.8	
$\overline{\mathbf{Cr}}$	0.2-1	0.5	
Cu	3-10	5.4	
${ m Fe}$	38-150	67	
Li	0.2-2	0.7	
Мп	16-30	21	
${f Mo}$	2-8	4	
Ni	1-4	1.7	
${ m Pb}$	0.2-2	1.1	
$\overline{\mathrm{Rb}}$	1-3	1.2	
Sn	0.04 - 1.5	0.5	
Sr	0.1-3	1.8	
${ m Ti}$	0.2-2	1.0	
Ÿ		0.1	
Zn	16-75	37	
Ag		0.05	
Be		0.5	
Bi		0.5	
Co		0.5	
Ga	aktonomi	0.1	
Ge	_	0.1	

After Pinta and Busson (1963).

 ${\bf T}_{\bf ABLE} \ 4.13$ average mineral composition of dry sorghum and sudangrass roughages

Mineral, $\%$	Sorghum Hay	Sudangrass Hay
Calcium	0.40	0.56
Phosphorus	0.17	0.31
Copper, Mg/lb	3.9	16.7
Potassium	1.4	1.5
Magnesium	0.32	0.4
Iron	0.005	0.017
Manganese, Mg/lb	52.5	42.3
Sulfur		0.06
Sodium	0.02	0.02
Chlorine	0.63	-
Cobalt, Mg/lb	******	0.06

National Academy of Sciences (1958).

indicate that potassium, the major mineral, makes up from 1.4 to 2.1% total dry matter. In general, various dry and green sorghum roughages have similar mineral compositions. However, limited data indicate that sudangrass contains more copper than does sorghum fodder.

Silica, calcium, and iron were determined in various parts of eight different hybrid sorghum plants (Lanning and Garabedian 1963). Roots contained 5.0% silica as compared to 4.0 and 2.9% in the sheath and leaves, respectively. Iron content (0.10%) of sorghum roots was 4 times higher than in leaves and sheath, both of which had nearly the same amount of iron. Calcium in the sorghum sheath (0.46%) was 2.5 times that of the leaves and 6 times that of the roots.

ENZYMES OF SORGHUM

To catalyze the chemical reactions necessary for its metabolism, the sorghum plant possesses many enzymes. Enzymes are generally saline-soluble proteins. For their activity they may require cofactors, such as metal ions or various low-molecular-weight organic substances.

Amylases

The most extensively studied enzymes of sorghum grain are those that degrade starch. Two kinds of amylases occur in plants. The α -amylase cleaves α -1,4 linkages at random. The β -amylases promote rapid hydrolysis of outer chains by breaking off 2 sugar units at a time (terminal maltose units), but they cannot hydrolyze or bypass α -1,6 linkages and, therefore, leave residual limit dextrans. Kneen (1944) found no β -amylases in ungerminated sorghum grain, but found small amounts of α -amylase as both free and bound enzyme. The bound α -amylase required protease activity to release it into solution. Sorghum grain had less α -amylase activity than oats, corn, or barley in the ungerminated state.

When allowed to germinate, sorghum grain exhibits a large increase in α -amylase activity (Kneen 1944). Germinated or malted sorghum grain serves as a source of enzymes for saccharification of starch before beverage fermentations as in preparation of native African beers. The sorghum α -amylase is stable to heating to 70°C. Its pH optimum from 20° to 40°C is 4.6 (Kneen 1945). It requires calcium ions for its activity. The α -amylase has been isolated from sorghum malts and purified by Dube and Nordin (1961) and by Botes *et al.* (1967A). Its molecular weight of 50,000 is similar to that of other α -amylases, and it contains 3 to 4 gm atoms of calcium per mole and 2% bound carbo-

hydrate. Paper chromatography of α -amylase digests of starch reveals that it forms mainly chains of 6 and 7 glucose units when the starch iodine color no longer appears, but as digestion continues, smaller oligosaccharides and maltose prevail (Dube and Nordin 1962).

In sorghum malt β -amylase accounts for only about 18% of the total amylase activity. Novellie (1960) concentrated it in malt extracts by ammonium sulfate fractionation. Ethanol fractionation and ion exchange chromatography were used by Botes *et al.* (1967B) to purify the enzyme further. The purified β -amylase has a pH optimum at 5.3 to 5.4. Only 55% of starch is converted to sugars by this enzyme.

Dyer and Novellie (1966) studied the distribution and activity of α - and β -amylases in germinating sorghum grain. Initially α -amylase is located mainly in the embryo, but as malting continues, both α and β activity exist in the endosperm as well as in the germ. The optimum development of the malt requires high moisture and a temperature of 25° to 30°C; maximum diastatic activity occurs at 6 to 7 days (Novellie 1962A). In addition to varietal differences of sorghums in malt quality, differences result from location and season of growth and storage conditions of grain (Novellie 1962B).

Many substances have been reported in grains that serve to inhibit amylase activities. Miller and Kneen (1941) isolated a high-molecular-weight organic acid from Leoti sorghum grain that reversibly inhibited barley malt amylase. The substance was not in most varieties tested and was concentrated mainly in the bran and germ.

Oxidative Enzymes

Enzymes have been investigated in sorghum grain and malt that catalyze oxidation of various substances. Peroxidase is an iron-porphyrin enzyme that promotes oxidation of substrates by hydrogen peroxide. Sorghum grain has less activity than sorghum after 7 days germination. Germinated sorghum exhibits much less activity than barley malt or grain (Reindel and Scheublein 1959). After 6 days germination, peroxidase activity increased rapidly in the sorghum embryo, according to Gopalachari (1963), with a smaller increase in the endosperm. Polyphenol oxidase, a copper-containing enzyme that oxidizes phenolic compounds, is absent in the mature grain but appears upon germination.

Hydrolases of Cyanogenic Glycosides

The importance of the cyanogenic glycoside, dhurrin, in young sorghum plants has stimulated studies on its enzymic synthesis and degradation. Homogenization of the plant results in rapid hydrolysis

of the compound to yield HCN. Dunstan and Henry (1902) demonstrated an enzyme that hydrolyzes the glycosidic linkage between glucose and p-hydroxybenzaldehyde cyanohydrin. Mao and Anderson (1967) have reported the isolation of two β -D-glucopyranosidases in sorghum vegetative tissues and seeds, but only one hydrolyzes dhurrin. The seeds have a higher concentration of enzyme than the plant. An enzyme that next decomposes the cyanohydrin of p-hydroxybenzaldehyde from etiolated seedlings of sorghum has been isolated also, purified, and designated hydroxynitrilyase (Seely $et\ al.\ 1966$). This enzyme has a pH optimum of 5 and is specific only for the L-isomer. The endosperm is inactive; greatest activity is in the developing epicotyl with lesser activity in the roots.

Sorghum seedlings have been widely used as enzyme sources in studies of many metabolic processes in plants, including photosynthesis and starch synthesis.

With the growing production of sorghum for forage and grain increased research is needed on its composition and metabolic processes.

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